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Research Article

Use of latrines and territorial marking behaviors by *Subulo gouazoubira* in a remnant of the Atlantic Forest in southeastern Brazil

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Abstract

The chemical communication signals the presence of individuals and territorial occupation, providing information about identity, sex, and reproductive status. This study aimed to characterize the use of latrines by the gray brocket deer, *Subulo gouazoubira*, in a remnant of the Atlantic Forest located in southeastern Brazil, as well as to describe its territorial marking behaviors, contributing to a better understanding of the species' social communication strategies. Four monitoring points were used, but latrine use was recorded at only two of them. Twenty-seven independent records of *S. gouazoubira* were obtained, totaling 29 specimens recorded, comprising 59% males, 28% females, and 14% deer with undetermined sex. Twenty-five interactions with latrines and five types of behavior were recorded: defecation, urination, front paw scratching against the ground, tail shaking, and sniffing the area near the latrine. Among the recorded behaviors, 56% were performed by females, 40% by males, and 4% by specimens with undetermined sex. There was variation in the types and frequency of behaviors recorded, indicating that territorial marking behavior might be variable between latrines. Latrine use also varied temporally concerning the time of day when behaviors were recorded and the interval between records of odoriferous marking at the same latrine. Differences in latrine use between males and females were also observed, suggesting intersexual variations in territorial marking strategy. The obtained results, although limited to a few sampling points, suggest complexity in territorial marking behavior and interaction with latrines in *S. gouazoubira*, highlighting spatial differences, distinct temporal dynamics, and variations between sexes.

Key words: Chemical communication, feces and urine deposition, intersexual interaction, intraspecific interaction, scratching

Introduction

The neotropical region boasts great habitat heterogeneity, providing varied niches that contribute to the vast species diversity (Tews et al. 2004). Among neotropical mammals, deer (Family Cervidae) encompass over 40 living species (Heckeberg 2020), with nine occurring in Brazil (Azevedo et al. 2021). In the Atlantic Forest, three genera of small, non-bifurcated, simple-antlered

deer (*Mazama*, *Subulo*, and *Passalites*) are currently recognized. Body size and fur coloration are significant traits in species classification within this group (Duarte et al. 2008; Azevedo et al. 2021; Bernegossi et al. 2022; Morales-Donoso et al. 2023). These species are traditionally divided into two clades: red brockets (*Mazama americana*, *Mazama jucunda*, *Mazama nana*, and *Mazama rufa*), from the Odocoileina subtribe, and gray brockets (*Subulo gouazoubira* and *Passalites nemorivagus*), from the Blastocerina subtribe (Duarte et al. 2008; Heckeberg et al. 2016). It is worth noting that these six species were previously grouped under the same genus, and only recently, based on morphological, cytogenetic, and genomic analyses, specimens previously classified as *Mazama gouazoubira* and *Mazama nemorivaga* were renamed as *S. gouazoubira* (Bernegossi et al. 2022) and *P. nemorivagus* (Morales-Donoso et al. 2023), respectively.

The gray brocket deer (*S. gouazoubira*) is a medium-sized cervid, weighing between 11 and 25 kg (Azevedo et al. 2021). Primarily solitary (Black-Décima 2000), it is found in Brazil, Uruguay, Argentina, Paraguay, and Bolivia (Black-Décima et al. 2010). The species inhabits various habitats, ranging from forests and savannas to cultivated areas (Azevedo et al. 2021). *Subulo gouazoubira*'s diet includes leaves, flowers, fruits, and seeds, classifying them as primarily frugivorous-granivorous regardless of the season (Gayot et al. 2004). Reproduction occurs throughout the year (Pereira et al. 2006), with a relatively long gestation period averaging seven months (Duarte 2014).

One of the social communication strategies employed by *S. gouazoubira* is chemical communication, often exhibited through the use of latrines (Black-Décima 2000; Black-Décima and Santana 2011). Latrines are specific sites where continuous odoriferous secretions, typically feces and at least one additional secretion, are deposited, serving both intra- and interspecific communication purposes (Darden et al. 2008). Chemical and aromatic communication occurs across various zoological groups, indicating an individual's presence and territory occupation (Roberts and Lowen 1997) or as a means to deter potential competitors (Bogoni et al. 2017). This form of communication happens when an individual alters its behavior upon sensing molecules produced and secreted by another individual through the air (Bossert and Wilson 1963). Thus, this communication can provide species-specific information (Caspers et al. 2009), as well as details about an individual's identity, sex (Linklater et al. 2013), or even its reproductive state (Ziegler 2013). The advantage lies in maintaining information at a location for an extended period, even when the depositing individual is absent (Mitro et al. 2012; Linklater et al. 2013). Specifically concerning reproduction, chemical information can aid in sexual selection, as females can determine a male's health status through these chemical cues (Kavaliers and Colwell 1995), providing insight into individuals' genetic quality (Charpentier et al. 2008). Consequently, reproductive periods tend to have a higher accumulation of feces at specific sites as part of the sexual communication strategy (Barja et al. 2011).

This manuscript aimed to characterize the use of latrines by *S. gouazoubira* in a remnant of the Atlantic Forest located in southeastern Brazil, as well as to describe the recorded territorial marking behaviors, contributing to a better understanding of the social communication strategies developed by the species.

Methods

Study area

The present study was conducted in the Linhares-Sooretama Block (Bloco Linhares-Sooretama—BLS; Fig. 1). The BLS, covering approximately 53,000 hectares, is situated in the northern region of Espírito Santo and spans across the municipalities of Linhares, Jaguaré, and Sooretama in southeastern Brazil (18.906404°S, 40.212993°W, and 19.243877°S, 39.946692°W). It comprises the Sooretama Biological Reserve (Reserva Biológica de Sooretama—RBS; 27,859 ha), the Vale Natural Reserve (Reserva Natural Vale—RNV; 22,711 ha), the Recanto das Antas Private Natural Heritage Reserve (Reserva Particular do Patrimônio Natural Recanto das Antas; 2,212 ha), and the Mutum Preto Private Natural Heritage Reserve (Reserva Particular do Patrimônio Natural Mutum Preto; 379 ha). The BLS represents the largest remnant of native vegetation in Espírito Santo, accounting for approximately 11% of the remaining forest remnants in the state (FSOSMA and INPE 2021).

The BLS encompasses a mosaic of various vegetation types, predominantly dense forest ("tabuleiro" forest), classified as perennial seasonal forest (Jesus and Rolim 2005). The region's climate is categorized as tropical with a dry winter (Aw type) according to the Köppen classification system (Alvares et al. 2014). The area experiences two climatic periods throughout the year: a dry season from April to September and a rainy season from October to March (Jesus and Rolim 2005; Kierulff et al. 2014). The average annual temperature in the region is 24.3 °C, ranging from 18.7 to 29.9 °C, and the average annual precipitation is 1,215 mm (Kierulff et al. 2014).

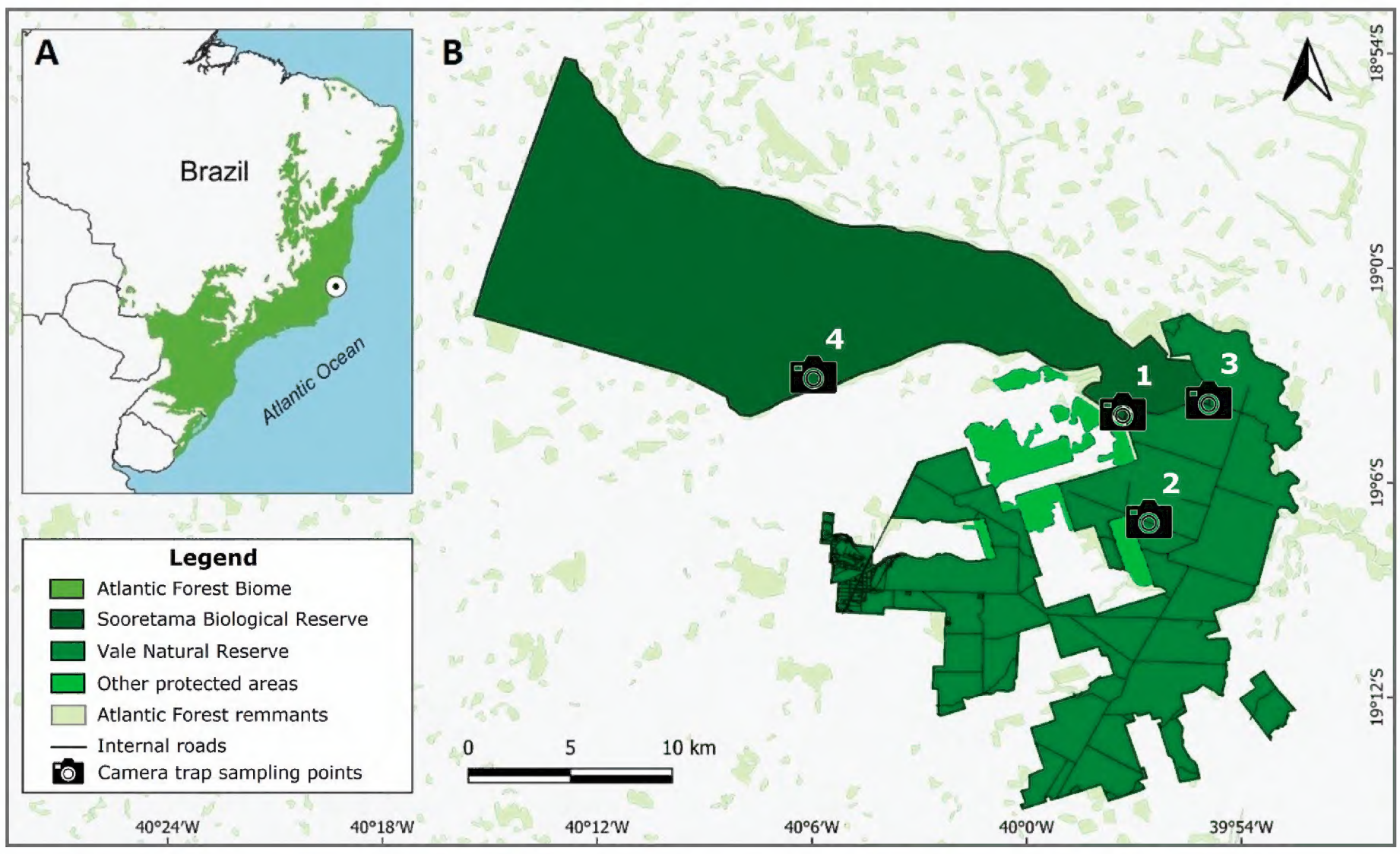

Figure 1. Location of the Linhares-Sooretama Forest Block in the northern part of the state of Espírito Santo, southeastern Brazil (**A**), including the limits of the protected areas that comprise it and other forest remnants present in the region, with emphasis on sampling points (**B**).

Data collection

Records of *S. gouazoubira* were obtained in the rainy season between October 2016 and February 2017 during a study to sample medium and large mammals in general. Digital camera traps, specifically the Bushnell Trophy Cam HD Aggressor Low-Glow model, were used. The equipment was set up along trails within the forest, and the records of the studied species were obtained at four distinct sampling points (one in RBS and three in RNV; Fig. 1). The average distance between sampling points was 11.7 km (range = 4.3 to 19.8 km). The equipment was configured to record videos lasting 10 seconds, with a 10-second interval between consecutive videos when there was continuity of movement. The camera traps remained active 24 hours a day without the use of bait.

Data analysis

For record counting (photos and videos), when multiple captures of the species were obtained within an hour at the same capture point, only the first record was considered valid (an independent record; Srbek-Araujo and Chiarello 2013). However, for behavior description, all obtained videos were considered. From the analysis of the records, a total of five behaviors were observed (a video with the observed behaviors is available in Suppl. material 1). Of these, three types of aromatic marking were identified: feces deposition ("defecating"), urine deposition ("urinating"), and front paw scratching against the ground ("scratching the ground"). In addition to these markings, interactions with latrines also included tail shaking ("shaking the tail") and sniffing the area near the latrine ("sniffing"). When none of these behaviors were observed, it was considered that the specimen simply passed through the location. The recorded specimens were classified as male, female, or undetermined sex (inability to confirm the presence or absence of antlers due to the specimen's position in the image).

Results

During the sampling period, 27 independent records of *S. gouazoubira* were obtained (totaling 29 specimens recorded), comprising 17 male records (59%), eight female records (28%), and four records of specimens with indeterminate sex (14%). Despite the records of *S. gouazoubira* being obtained at all four sampling points, the use of latrines was detected at only two of these points. A total of 25 interactions with the latrines (points 1 and 2) were documented, with "sniffing" being the most common behavior (n = 10; 40%), followed by "defecating" (n = 5; 20%), and "urinating" (n = 5; 20%). The categories "scratching the ground" (n = 3; 12%) and "shaking the tail" (n = 2; 8%) were the least recorded. The behaviors were observed separately or in association with other behaviors. Among the observed behaviors, 56% (n = 14) were performed by females, 40% (n = 10) by males, and 4% (n = 1) by specimens with undetermined sex. Males exhibited all five behavioral categories, with "sniffing" being the most recorded (n = 4; 40%), followed by "defecating" and "shaking the tail" (n = 2; 20% each), while "urinating" and "scratching the ground" (n = 1; 10% each) were the least recorded. Among females, four behaviors were recorded, with "sniffing" being the most frequent (n = 5; 36%), followed by "urinating" (n = 4; 29%), "defecating"

(n = 3; 21%), and "scratching the ground" (n = 2; 14%). The behavior of "shaking the tail" was not observed in females. One of the specimens with undetermined sex was observed "sniffing."

At sampling point 1, 10 independent records were documented (4 males and 7 females). All deer were observed in solitary activity without direct interaction with other specimens. On one occasion, a female and a male were captured with a few minutes between records. The first record at point 1 was on 27 October 2016, at 16:17 h, where a female was observed initially defecating while standing and then squatting (probably to urinate). During this event, the tail was raised entirely, exposing the entire white-colored underside. After defecation, the specimen calmly walked away while sniffing the path as it moved away from the latrine. Three days later, on 30 October 2016, at 12:06 h, the second record occurred. A female was observed feeding on leaves and continued sniffing until reaching the latrine. Using the left front paw, the specimen scraped the ground three times, repeating the movement with the right front paw. Immediately after this action, the female squatted and urinated on the spot. On 03 November 2016, at 07:33 h, a male was recorded passing through this point. The specimen rapidly wagged its tail in short intervals as it moved away from the camera trap. Less than 24 hours later, on 04 November 2016, at 05:20 h, a female was recorded eating leaves. Afterward, she sniffed until she reached the location where another female had urinated five days earlier. She intensely sniffed the spot, then squatted and urinated. On the same day, at 08:32 h, a male passed, sniffing and wagging its tail as it distanced itself from the latrine. On 08 November 2016, at 13:32 h, a female passed by the point, and 16 minutes later, a male was observed moving a bit faster while sniffing in the direction of the female. On 15 November 2016, at 07:56 h, a female was observed scraping its paws on the ground. This specimen scraped at least once with the right front paw and three times with the left front paw. Afterward, she squatted with the tail raised and urinated. Following urination, the female stood up and briefly sniffed the ground in front of her. Similarly to a previous record at this point, on 24 November 2016, at 18:25 h, a female was feeding on leaves and, upon arriving at the latrine, began to defecate while initially standing and then squatting, continuing to defecate with the tail raised. On 02 December 2016, at 06:30 h, a male was observed eating leaves where other specimens had been observed feeding. After sniffing the latrine area, he squatted to urinate. Twelve days later, on 14 December 2016, at 22:18 h, a female arrived sniffing at the location. She stayed alert for a moment and continued sniffing until reaching the latrine. At 22:20 h, she started defecating while standing with the tail raised, followed by squatting and urinating at 22:21 h. No additional records of the species were obtained at this sampling point until the end of the sampling period.

At point 2, 15 independent records were obtained (11 males, 1 female, and 4 sex unidentified), highlighting the recording of a male and a female together in the same event. At this point, the first record occurred on 23 October 2016, at 14:47 h. A male was observed arriving at the site and scratching its right front paw on the ground five times. Before repeating the same gesture three times with the left front paw, the specimen approached the dug-up area and visibly sniffed the left odor. Five days later, on 28 October 2016, at 07:25 h, a sex-undetermined specimen was recorded running through this point. The following day (29 October 2016), at 07:18 h, a male only passed through the location. In the

early hours of 01 November 2016, at 04:02 h, a male calmly passed through the area, coming from the direction of the latrine. On 05 November 2016, thirteen days after the first record, a male was observed defecating at the previously dug-up location. Within a month after this last event, three males and a specimen with undetermined sex were recorded merely passing through the site, all between 04h and 06h in the morning. On 08 December 2016, at 06:17 h, another male was recorded sniffing toward the latrine and defecating on it. On 02, 06, and 11 January 2017, two males and one sex unidentified specimen were recorded passing through the location at different hours. On 22 January 2017, at 12:02 h, a female was observed walking slowly through the location, with a male walking in the same direction nearby. On 11 February 2017, at 05:29 h, the last record occurred, in which a specimen with unidentified sex was observed passing through the area while sniffing.

Only one male was recorded at point 3 and another male at point 4. The first was observed urinating in October 2016, at 07:30 h, and the other defecating in January 2017, at 00:41 h. However, these locations were not classified as latrines since there were no sequential records suggesting the repeated deposition of odoriferous signals at these points.

Discussion

The use of latrines by *S. gouazoubira* was confirmed for the BLS, with three types of aromatic marking observed among the specimens. Besides marking by feces and urine, the behavior of front paw friction on the ground was also noticed. Latrine maintenance using paw friction, feces deposition, and urine had been reported previously for *S. gouazoubira* under semi-captive conditions (Black-Décima 2000). However, paw friction had been observed in association with pressing the forehead against branches or bushes in the earlier study (Black-Décima 2000), a behavior not observed in the present study. *Subulo gouazoubira* possesses odoriferous glands, typically composed of sebaceous and/or sudoriferous glands, in the orbital, frontal, and interdigital regions (Ajmat et al. 2004). This explains head and paw friction as elements of chemical communication. The gathered records, along with those of Black-Décima (2000), suggest that the three behaviors described in the BLS might represent typical odoriferous marking methods of *S. gouazoubira*, although variations in the behavioral repertoire may exist.

As observed in this study, it was noted that, regardless of sex, *S. gouazoubira* squatted while keeping the tail raised when defecating and urinating (Black-Décima 2000). The species possesses highly developed sebaceous and sudoriferous glands associated with the tail, suggesting that these glands serve as an important source of pheromones (Ajmat et al. 2004). Indeed, the caudal region of *S. gouazoubira* appears to have an important communicative function, as it has been observed that when two individuals meet, this region is subject to mutual sniffing. Along with this behavior, individuals also repeatedly move their tails (Ajmat et al. 2004). In this study, males were recorded swiftly and briefly moving their tails when passing near the latrine on two occasions, although they were not observed defecating or urinating on it during the same capture event.

The records obtained at the BLS indicate that the behavior of sniffing upon approaching latrines is common in *S. gouazoubira*, irrespective of sex. Experiments

involving the introduction of feces from different individuals into distinct latrines led to the perception that these animals "investigate" the odors of unknown individuals (Black-Décima and Santana 2011). Both males and females recognize their own aromatic marks, while the scent of unfamiliar individuals triggers the act of sniffing (Black-Décima and Santana 2011). Furthermore, the detection of feces or urine from other individuals of the same sex induces the act of defecation and urination in the same latrine or nearby, serving to reaffirm their presence, which may indicate a response to intrasexual competition (Black-Décima and Santana 2011). In the BLS, the latrine with the highest frequency of odoriferous marks also accumulated the greatest number of specimens sniffing.

The records of *S. gouazoubira* using latrines in the BLS did not occur at regular time intervals, as observed in both points 1 and 2. It was noted that the interval between odoriferous marking records varied from less than 24 hours to over a month at the same latrine. Regarding the temporal aspect, it is worth mentioning that deer engaged in urination and/or defecation during all periods of the day (morning, afternoon, and night), but paw friction behavior against the ground was not documented during the nighttime.

Due to the impossibility of differentiating individuals of the same sex, it can be asserted that latrine use was performed by at least two individuals at both latrines, as males and females were recorded at both points. However, at point 1, more marking records were obtained, with females contributing more frequently to latrine maintenance than males, while at point 2, only males were confirmed to engage in such activities. This suggests that territorial marking behavior and latrine use may vary between latrines within the same population regarding overall usage intensity and intensity between sexes.

In the present study, a similar number of records were obtained of specimens defecating and urinating when both latrines were considered together. However, at point 1, urine deposition was more frequent and performed mainly by females, while at point 2, there was more fecal deposition, exclusively by males. Feces, in general, release aromatic compounds more gradually than urine, emitting signals over a longer period of time (Vilà et al. 1994). Additionally, feces persist longer in the environment as urine evaporates easily (Barja and List 2006). Nevertheless, it should be considered that urine might also play an important role in olfactory communication in *S. gouazoubira*. For example, in canids, urine has a more specific informative function, highlighting that latrines in this group are primarily constituted by urine, potentially conveying various characteristics simultaneously (Allen et al. 1999; Ralls and Smith 2004). Moreover, in rhinoceroses, the estrus signal of females is present in urine, with urine being more important than feces for transmitting this reproductive signal (Owen-Smith 1975). Therefore, the fact that females urinated more frequently at point 1 might be related to intersexual variations in territorial marking strategy or an intensification of urine marking during the estrus period, which might have coincided with the sampling period.

The presented information aligns with other studies by considering that latrines serve as a means of intersexual communication among individuals sharing the same space (e.g., Wronski et al. 2006; Black-Décima and Santana 2011). In this context, the use of latrines by individuals of both sexes and the recording of a female and a male together or recorded with a small-time interval in the present study reinforce that the use of latrines by *S. gouazoubira* might be associated with reproductive communication in the BLS.

Besides the reproductive purpose, the use of latrines might also be related to communicating the boundaries of territories between females and males, informing neighbors and potential intruders that the area is occupied by an individual (e.g., Zollner et al. 1996; Black-Décima and Santana 2011). Hence, latrines might be distributed around an individual's home range, where continuous defecation and urination occur (Black-Décima and Santana 2011). However, this behavior might vary depending on sex. Specifically, male *S. gouazoubira* more frequently marks outside and at the boundary of the territory, while females mark within their home ranges (central areas of the home range are defended as small territories) as a form of social communication within the group (Black-Décima 2000). During a study with gazelles, latrines located in male territory overlap zones were almost exclusively used by males, indicating that the deposited signals stimulate responses from individuals of the same sex, considering territoriality (Wronski et al. 2013). Conversely, in female territorial overlap zones, latrines were also used by males (Wronski et al. 2013). It is possible that the behavior observed for *S. gouazoubira* is similar, since in point 1, both females and males contributed to the latrine, unlike point 2, where only males deposited feces and urine or did paw friction. It is known that female *S. gouazoubira* have smaller home ranges and territories, with some territories presenting a high overlap rate with males. On the other hand, males have larger territories than females (Black-Décima 2000; Black-Décima and Santana 2011). During territorial overlap events (both male and female *S. gouazoubira* are territorial), the presence of individuals of the same sex may trigger aggressive behavior, unlike encounters with individuals of the opposite sex, where receptive behavior is observed (Black-Décima 2000). Thus, we agree that the use of latrines by *S. gouazoubira* might represent an important strategy to prevent unwanted encounters and avoid agonistic interactions between specimens.

Conclusions

Subulo gouazoubira displays complex territorial marking behavior and interaction with latrines, which may vary between latrines and demonstrate distinct temporal dynamics, as well as intersexual variations. It is important to emphasize that understanding the significance of latrines as elements of communication and social interaction for a particular species is essential for the development of efficient monitoring and conservation strategies, incorporating aspects of habitat, physiological characteristics, and behavioral traits of the target species.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Srbek-Araujo, A.C.: Funding acquisition, Conceptualization, Investigation, Methodology, Data curation, Formal Analysis, Validation, Visualization, Writing – original draft. Alzuguir, L.C.: Writing – original draft.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Behaviors recorded for the gray brocket deer (*Subulo gouazoubira*) in the Linhares-Sooretama Block: aromatic marking (defecating, urinating, and scratching the ground) and other interactions with latrines (shaking the tail and sniffing)

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